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Microwave-assisted synthesis, molecular docking and antitubercular activity of 1,2,3,4-tetrahydropyrimidine-5-carbonitrile derivatives

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ABSTRACT

Based on bioisosteric similarities with isoniazid, a series of 1,2,3,4-tetrahydropyrimidine-5-carbonitrile derivatives has been designed. The target compounds have been synthesized by multicomponent reaction which involves one-pot organic reactions using ethylcyanoacetate, urea/thiourea and arylaldehydes in presence of ethanolic K_2CO_3. Two methodologies, conventional and microwave-assisted, have been adopted for the synthesis. The later strategy gave high yields in less than 10 min as compared to long hours using the former approach. Molecular docking of the target compounds into the enzyme *Mycobacterium tuberculosis* enoyl reductase (InhA) revealed important structural information on the plausible binding interactions. Major binding interactions were found to be of dispersion type (residues Tyr158, Ile215, Met103 and Met199) and a hydrogen bond with Tyr158. Binding poses of the all the compounds were energetically favorable and showed good interactions with the active site residues. Few selected compounds were also evaluated for antitubercular activity in vitro against drug-sensitive *M. tuberculosis* H37Rv strain and clinically isolated S, H, R and E resistant *M. tuberculosis* by luciferase reporter phage (LRP) assay method. Some compounds displayed promising antimycobacterial activity comparable or less than the standard drugs isoniazid and rifampicin.

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Mycobacterium tuberculosis (MTB) is the underlying microorganism causing tuberculosis (TB) in humans. It is considered as the leading bacterial infectious agent.[1] Tuberculosis usually attacks the lungs but can also affect other parts of the body. It spreads through the air by patient's cough, sneeze, or spit.[2] India accounts for nearly one-third of the global burden of tuberculosis and the disease is one of India's most challenging public health problems. Approximately 2 million people acquire TB every year in India.[3] Furthermore, it is alarming to see the emergence of MTB strains resistant to all of the first line drugs (leading to the multi drug resistant TB, MDR–TB) and to isoniazid, rifampin, fluoroquinolone and at least one of three injectable second-line drugs (i.e., amikacin, kanamycin, or capreomycin) (leading to the extensive drug-resistant TB, XDR–TB).[4] All the above facts reveal that there is an urgent need for the development of new potent drug molecules with unique divergent structure and novel mode of action which can be effective against resistant strains of mycobacteria. Isonicotinic acid hydrazide (isoniazid, INH) belongs to the group of the first line antitubercular drugs being in clinical practice over 50 years. INH is believed to kill mycobacteria by inhibiting the biosynthesis of mycolic acids, critical component of the cell wall.[5] Thus, it was thought of interest to replace the pyridine ring of INH with its pyrimidine bioisostere (Scheme 1). This bioisosteric replacement followed molecular docking of the designed compounds into crystal structure of *Mycobacterium tuberculosis* enoyl reductase (InhA) (PDB Code 2H7I).[6] The present enzyme was chosen over a large number of other enoyl reductases as it has better resolution (1.62 Å) over many others (>2.0 Å). Compounds which were found promising in the docking study were evaluated for their antimycobacterial activity by luciferase reporter phage (LRP) assay method against *M. tuberculosis* H37Rv and clinically isolated S, H, R and E resistant *M. tuberculosis* at two concentrations (100 and 500 μg/ml).

Multi-component reactions (MCRs) constitute a highly valuable synthetic tool for the construction of polyfunctionalized heterocyclic compounds required for drug discovery programmes.[7,8] Thus, a series of 1,2,3,4-tetrahydropyrimidine-5-carbonitrile derivatives were synthesized based on multicomponent reaction (MCR) which involves one-pot organic reactions (Scheme 1).[9] This reaction

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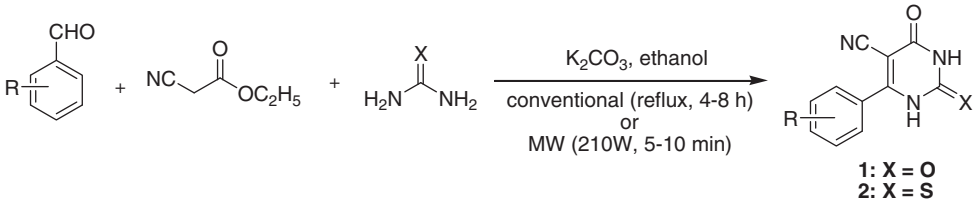
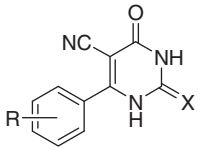


Scheme 1. Synthetic route for the synthesis of title compounds.

Table 1
Chemical structures and physical properties of target compounds



Compd. No.	X	R	mp (°C)	Yield (%)		Time (min)	
				C[a]	M[b]	C	M
1a	O	H	275–280	61	83	240	5
1b	O	4-F	255–260	61	81	360	7
1c	O	2-Cl	290–295	60	81	300	6
1d	O	3-Cl	223–228	59	79	420	8
1e	O	4-Cl	235–241	61	82	360	7
1f	O	3-Br	277–281	57	78	300	7
1g	O	4-Br	285–288	58	80	360	9
1h	O	4-OH	290–293	57	79	480	10
1i	O	3-OH,4-OCH_3	248–252	56	77	480	8
2a	S	H	285–287	66	85	240	5
2b	S	4-F	195–200	65	85	360	9
2c	S	2-Cl	185–190	63	82	300	7
2d	S	3-Cl	275–280	61	80	420	8
2e	S	4-Cl	205–208	61	83	360	6
2f	S	3-Br	250–252	58	79	300	7
2g	S	4-Br	242–245	59	81	360	8

[a] C — Conventional.
[b] M — Microwave.

involves two mechanisms such as Knoevenagel condensation and Michael addition. First, various arylaldehydes react with ethylcyanoacetate in presence of ethanolic K_2CO_3 to produce an intermediate by Knoevenagel condensation reaction. Then, the intermediate reacts with urea/thiourea via Michael addition to produce corresponding pyrimidine derivatives.[10,11]

Table 1 represents the summary of all the compounds synthesized. Clearly, the microwave approach proved to be fast and clean. The yields were found to be quite high (75–85%) as compared to conventional synthesis (55–65%). We believe that the most noticeable advancement was the speed with which the reaction proceeded. Reactions under microwave were completed within just 5–10 minutes, being 50–70 times faster than the conventional methodology.

Next, we were interested to investigate the biological profile of the target compounds. Considering reasonable structural similarity of the target compounds with the blockbuster antitubercular agent INH, we decided to dock the target compounds into the active site of the molecular target of INH, *Mycobacterium tuberculosis* enoyl reductase (InhA). First, in order to identify proper docking protocol, we independently used two different programs: Dock 6.5[12] and Glide.[13] We docked the native ligand of *Mycobacterium tuberculosis* enoyl reductase (PDB code 2H7I) and investigated the root mean square deviation (rmsd) between the crystal geometry and the docked pose. While both the docking programs gave satisfactory rmsd values (<2 Å), Glide pose was better (rmsd = 0.47 Å, Fig. 1a). Hence, we used Glide XP protocol for docking the target compounds. Since, all the target compounds contained the same core moiety (1,2,3,4-tetrahydropyrimidine-5-carbonitrile) with very little modifications on the phenyl rings, the binding energy (Glide-XP-Score) were found to be very similar (−5 to −6, Table s1, Supplementary data). We know from the crystal geometry of 2H7I ligand (1-cyclohexyl-5-oxo-N-phenypyrrolidine-3-carboxamide) that the interactions are chiefly of dispersion type. The ligand is buried inside a hydrophobic pocket and is less exposed to solvent. The cavity is made up of some nonpolar amino acids like Tyr158, Ile215, Met103 and Met199. The ligand also makes a hydrogen bond with Tyr158. All these interactions are shown in Figure 1a. Next, we analyzed the binding interactions of the target compounds with the neighboring residues. It was encouraging to see that the ligand occupied essentially the same pocket and preferentially also retained all the interactions mentioned above with some additional interactions. For example, compound **2g** showed an additional interaction, that is, halogen bond with backbone oxygen of Pro156 (Fig. 1b). However, INH had a very low score (−3.6). Hence, we thought of rationalizing the low score of INH. Figure 1 shows the fit of **2g** (panel c) and INH (panel d) into the active site of the enzyme, which suggest that the target compounds better fit the cavity as compare to a INH, being much smaller in size.

After satisfactory results of the docking of target molecules into the *Mycobacterium tuberculosis* enoyl reductase enzyme, we selected a few candidates (which were found potent in silico) for the evaluation of the antimycobacterial activity. LRP assay is a rapid, inexpensive and less laborious method for high throughput screening of compounds for their antimycobacterial activity.[15] A compound is considered to be an antimycobacterial agent if 50% reduction in the Relative Light Units (RLU) is observed when compared to the control using a luminometer.[16,17] Results of the antimycobacterial activity are summarized in Table 2.

In summary, we designed a series of 1,2,3,4-tetrahydropyrimidine-5-carbonitrile derivatives and synthesized them using conventional and microwave-assisted one pot multicomponent synthesis. Microwave-assisted synthesis proved to be a better synthetic approach due to better yields and short reaction times. In order to investigate how the target compounds bind to target, they were docked into one of the plausible target *Mycobacterium tuberculosis* enoyl reductase. Most of the target compounds occupied energetiacally more favorable position in the active site cavity than isoniazid. However, none of the compounds (although few being decently active) was found to be better in their in vitro antimycobacterial activity as compared to isoniazid, when tested using luciferase reporter phage assay against *M. tuberculosis* H37Rv and clinical isolates S, H, R, and E resistant *M. tuberculosis*. The inaccuracy to predict isoniazid as potent inhibitor of *M. tuberculosis* enoyl reductase in silico by docking than the target compounds might be due to the well-known issues with the docking (sampling and forcefield-based scoring function) or different molecular target of the synthesized compounds, or both.

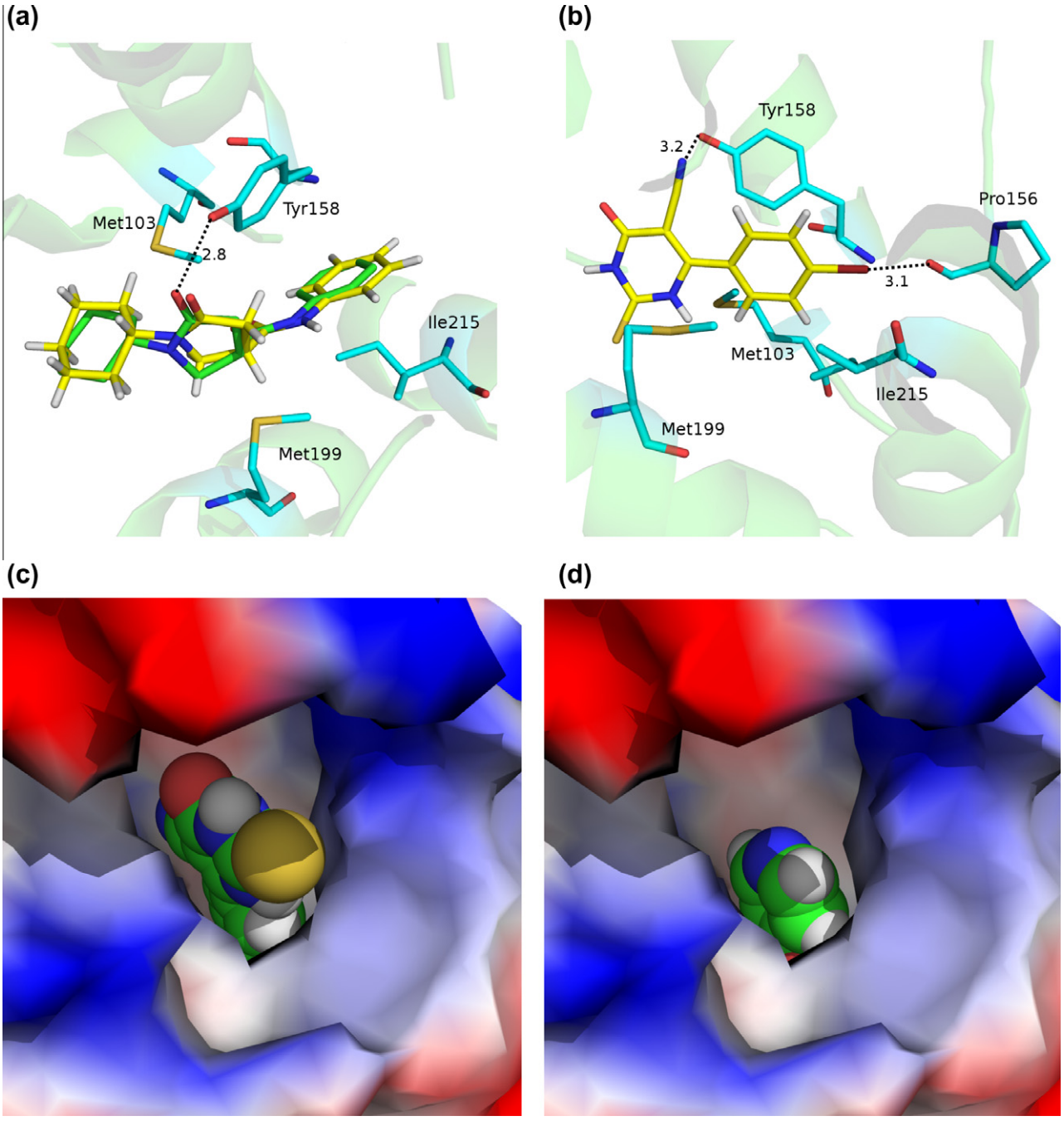


Figure 1. A close-up view of the binding interactions of the docked ligands in *Mycobacterium tuberculosis* enoyl reductase (PDB code 2H7I).[6] (a) Overlay of the native ligand with the crystal structure; (b) compounds **2g**. The fit of the compounds **2g** (c) and INH (d) (spheres) in the active site. Color coding: Ligand carbon in yellow (docked geometry) or green (crystal geometry), protein carbon in cyan, nitrogen in blue, oxygen in red, bromine in brown, sulfur in light brown, hydrogen in white. The figure was prepared using Pymol, ver. 0.99.[14]

Table 2
Antimycobacterial activity of the target compounds

Compd. No.	%Reduction in RLU			
	M. tuberculosis H37Rv		Clinical isolate: S, H, R and E resistant *M. tuberculosis*	
	100 μg/mL	500 μg/mL	100 μg/mL	500 μg/mL
1b	46.49	44.20	38.38	47.86
1d	54.28	61.02	50.10	52.77
1i	33.40	48.72	25.47	33.49
2e	46.21	65.23	61.35	66.65
2g	75.73	82.04	38.03	74.91
Rifampicin (2 μg/mL)	82.58		29.19	
Isoniazid (0.5 μg/mL)	98.42		89.6	

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.10.032.

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